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STIMULATION OF SPAWNING IN THE MUSSELS, MYTILUS EDULIS LINNAEUS AND MYTILUS CALIFORNIANUS CONRAD, BY KRAFT MILL EFFLUENT[1]

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Various procedures have been employed by different investigators to stimulate spawning in the bay mussel, *Mytilus edulis* Linnaeus, and the California sea mussel, *M. californianus* Conrad, in the laboratory. These methods have met with varied success in terms of ease, efficiency, and reproducibility. Field (1922) claimed that he was able to induce spawning in the bay mussel within an hour by rough handling, such as shaking the animals in a bucket. Costello *et al.* (1957), however, were unable to stimulate spawning in the bay mussel by this treatment. The latter authors were able to obtain gametes, if the gonads were fully ripe, by removing the valves of an animal and placing the mantle folds in a small bowl containing about 100 cc. of sea water. Just (1939) was able to obtain gametes from the bay mussel by placing intact animals in bowls with sea water and allowing natural spawning to occur. Similarly, Berg and Kutsky (1951) obtained gametes from the bay mussel by allowing natural spawning to occur in bowls of sea water following storage of the animals in a dry state in a refrigerator at 4° C. Under these conditions spawning occurred up to one week after storage. However, it was not clear from these reports how readily spawning occurred, or how efficient was the method.

Iwata (1950a, 1950b; 1951a, 1951b, 1951c, 1951d, 1951e, 1951f, 1951g; 1952) studied the stimulation of spawning in *M. edulis* under more precisely controlled conditions. He found that spawning occurred in intact animals within 30–60 minutes after an electrical stimulation of 20 volts for 5 seconds, or within 1–5 hours after an injection of 0.5 cc. of *M*/2 KCl, but not sea water, into the mantle cavity. Spawning also occurred within 30–90 minutes if mantle pieces were bathed for 5–10 minutes in *M*/2 KCl, *M*/2 NH_4Cl, or *M*/3 $BaCl_2$; stimulated electrically; or sub-

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jected to a sudden temperature rise from 7° to 15° C; *M*/2 NaCl, *M*/2 LiCl, *M*/3 $SrCl_2$, *M*/3 $CaCl_2$, and *M*/3 $MgCl_2$ did not induce spawning from mantle pieces. Ova obtained under these conditions were mature and were fertilized by sperm obtained in the same manner.

Sugiura (1962) has also shown that excised mantle pieces from hermaphroditic *M. edulis* can be induced to spawn by electrical stimulation.

In the case of the sea mussel, Young (1945) reported that very few animals would spawn spontaneously in the laboratory either as a result of exposure to air or by transfer from one container to another. Likewise, a gradual or sudden rise in temperature was ineffective in inducing spawning. However, mechanical stimulation, such as scraping the shells to remove adherent organisms or pulling the byssus, or sexual stimulation were very effective in inducing spawning within 1–6 hours following stimulation. The latter method involved the placing of spawning mussels into containers with the ones to be tested, or introducing freshly deposited spawn or macerated gonadal tissue into the containers holding the test animals. Very few control animals, maintained in sea water in bowls, spawned after 10 hours, but the majority of the experimental animals did so following stimulation by either of the above methods.

In view of the variability encountered with the various methods used for the stimulation of spawning in mussels, it was deemed advisable to present in detail the results of experiments performed monthly during 1959–1963 on the stimulation of spawning in the bay mussel by kraft mill effluent (sulfate process pulp mill waste). The experiments on the sea mussel were carried out only on animals collected during January and February, 1963. The present report is concerned with these findings.

Materials and Methods

Bay mussels were collected from pilings in Yaquina Bay, and Alsea Bay, Oregon, and sea mussels from rocks from a protected outer coast at Seal Rock, and at Otter Rock, Oregon. The animals were scraped free of adherent organisms on the day of collection, maintained in a dry state overnight at room temperature, and used the following day.

Experiments on intact animals were carried out in small fingerbowls, with one mussel per bowl. Control mussels were maintained in 200 cc. of fresh sea water, and the test mussels in 200 cc. of 4% kraft mill effluent (KME), diluted by volume with sea water, obtained from a local paper mill. In some experiments, the stimulatory activity of KME was also tested by removing one-half of the control animals from the sea water after 4–5 hours and placing them in KME.

Animals were subjected to an electrical stimulation of 16 volts alternating current for 15 seconds by carefully separating the valves sufficiently to permit the insertion and placement of the electrodes in contact with the mantle. Following stimulation the mussels were maintained individually in 200 cc. of sea water in finger bowls. Controls consisted of unstimulated and sham operated animals. Four hours after stimulation, mussels in all three groups which had not spawned were tested for reactivity to KME by placing them in 200 cc. of a 4% dilution of this material.

All experiments were conducted at approximately 20° C. Following stimulation the animals were observed for 24 hours for the emission of gametes.

To test for maturity of ova and viability of sperm following KME stimulation, several mussels in each experiment were removed from the KME solution at the first sign of spawning, washed, and placed in clean sea water. Under these conditions the animals continued to spawn and the gametes so obtained were harvested and used in fertilization tests.

Results

The results of experiments on the stimulation of spawning in mussels by KME are summarized in Table I. It is clear that KME has a marked stimulatory

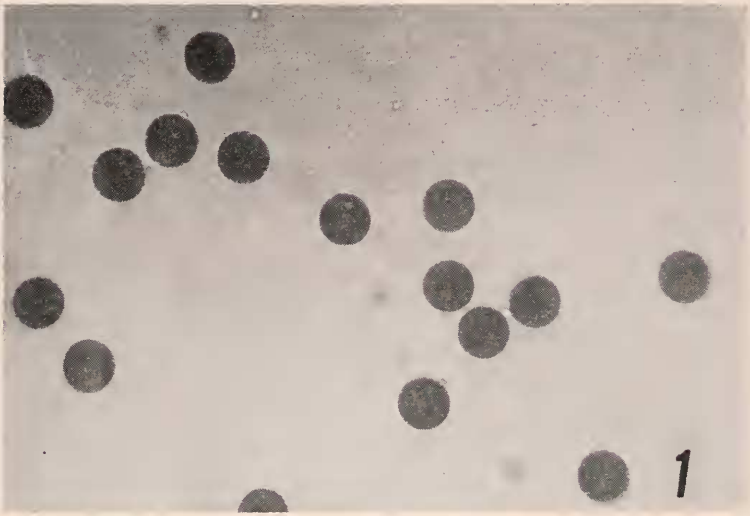
Table I

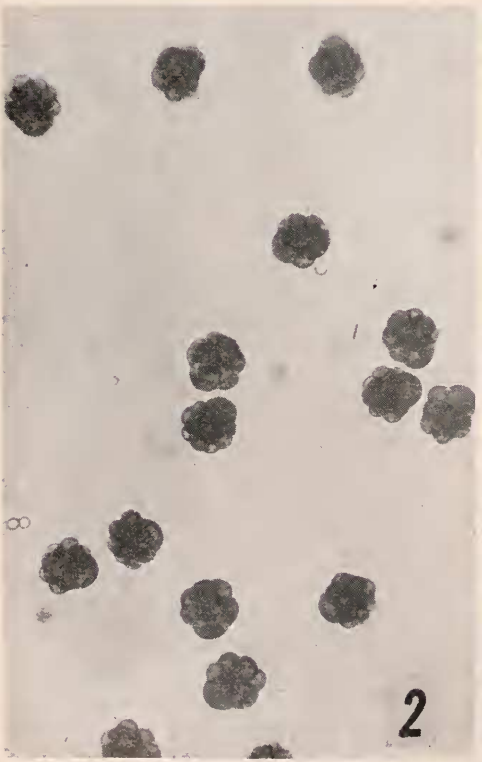
Effect of kraft mill effluent (KME) on spawning in Mytilus edulis *and* M. californianus

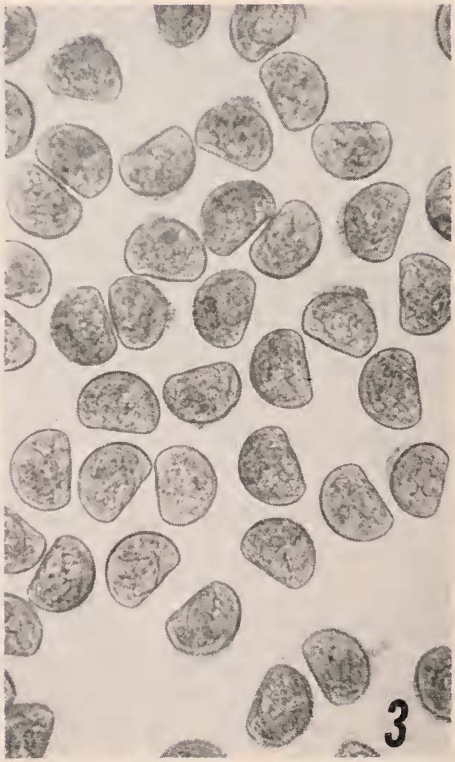
Exp. No.	KME*		Control		Control + KME**		
	No. spawned/ No. tested	% Spawned	No. spawned/ No. tested	% Spawned	No. spawned/ No. tested	% Spawned	
						Before KME	After KME
M. edulis							
1	19/29	66	2/30	7			
2	6/30	20	1/30	3			
3	17/30	57	1/30	3			
4	17/30	57	0/30	0			
5	18/30	60	2/30	7			
6	19/30	63	1/30	3			
7	31/90	34	0/30	0			
8	24/30	80	0/18	0			
9	50/60	83	1/28	4			
10	4/30	13	0/30	0	7/30	0	23
11	10/30	33	1/30	3	11/30	0	37
M. californianus	2/30	7	0/30	0	4/30	0	13

* 4% KME in 200 cc. of sea water.
** Animals placed in KME after 5 hours in sea water.

activity on mussels, whereas very few of the control animals, maintained in sea water, spawned during the 24-hour observation period. Further evidence in support of the stimulatory activity of KME is seen in experiments 10, 11, and 12, where mussels were placed in KME after 5 hours in sea water. Under these conditions no mussels spawned before but many did so after stimulation. The reaction time, the time between the application of the stimulus and the first appearance of gametes, in these experiments varied between 20 minutes and 24 hours, with 41% of the animals spawning within the first hour. There was no sexual difference in responsiveness to KME. Approximately equal numbers of males and females spawned in each experiment. The gametes obtained by KME stimulation were

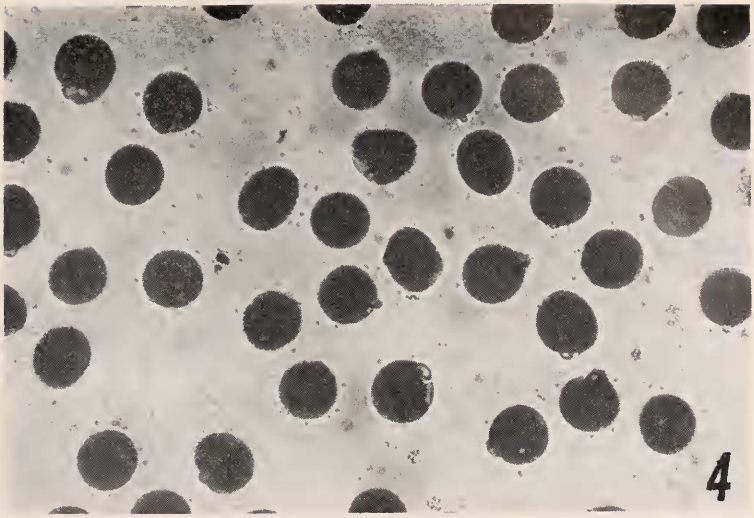


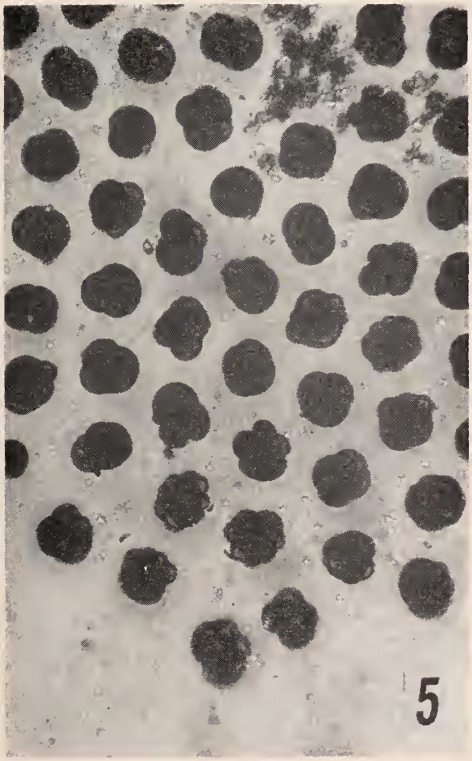


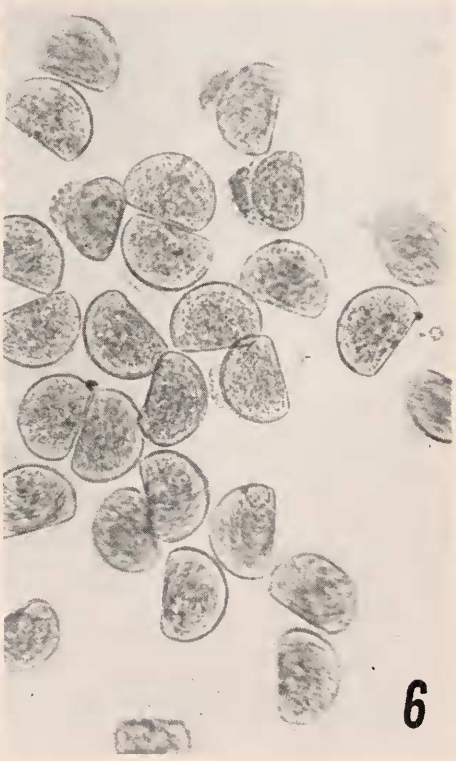


Development of *M. edulis* following artificial fertilization of gametes obtained from mussels stimulated with KME. × 100.

FIGURE 1. First polar body, 40 minutes.
FIGURE 2. Eight-sixteen-cell stage, 200 minutes.
FIGURE 3. Shell stage, 48 hours.







Development of *M. californianus* following artificial fertilization of gametes obtained from mussels stimulated with KME. × 100.

FIGURE 4. First polar body, 75 minutes.
FIGURE 5. Four-eight-cell stage, 165 minutes.
FIGURE 6. Shell stage, 48 hours.

viable and capable of fertilization (Figs. 1–6). The approximate times, for both species, for the appearance of the first polar body, 2–4-cell stage, swimming stage, and shell stage at room temperature were 40–75 minutes, 90 minutes, 18 hours, and 48 hours, respectively, after fertilization.

In Table II are summarized the results of experiments on spawning of mussels by electrical stimulation, as well as by KME stimulation. The evidence suggests that electrical stimulation *per se* may be effective in triggering spawning, although some of the sham operated controls also spawned. Of interest was the finding that animals which did not respond to electrical stimulation or to the sham opera-

TABLE II

Effect of kraft mill effluent (KME) and electrical stimulation on spawning in Mytilus edulis *and* M. californianus

KME		Electricity		Sham Controls*		Controls	
No. spawned/No. tested	% Spawned	No. spawned/ No. tested	% Spawned	No. spawned/ No. tested	% Spawned	No. spawned/ No. tested	% Spawned
M. edulis 15/25	60	9/25	36	4/25	16	2/25	8
		After KME Stimulation**					
		5/16	31	14/21	67	8/23	35
M. californianus 8/25	32	4/25	16	2/25	8	0/25	0
		After KME Stimulation					
		0/21	0	2/23	9	3/25	12

* Not stimulated electrically but otherwise treated in an identical manner.
** Non-spawning animals exposed to 4% KME after 4 hours.

tion were capable of subsequent stimulation by KME, although more of the animals in the latter group did so.

DISCUSSION

The results of the experiments reported here demonstrate that KME is a highly effective stimulus in triggering spawning in the bay and California sea mussels. Moreover, this material will stimulate spawning in bay mussels obtained during all times of the year, although to a somewhat lesser extent in animals collected during the months of October through December. This has been repeatedly confirmed in this laboratory in many other tests in which KME was used to trigger spawning in bay mussels in order to obtain gametes for use in bio-assay tests on the toxicity of various concentrations of mill effluents to developing mussel embryos.

Thus, the technique described here to induce spawning is simple, efficient, and affords a high degree of reproducibility. In addition, stimulation of spawning by KME does not affect the viability and fertilization capacity of the gametes, since the ova and sperm so obtained are fully capable of fertilization with development of the embryos to the shell stage.

The results reported here on the stimulation of spawning in mussels by KME complement the observations of others that certain chemicals are very effective in this regard. Iwata (1951f) has demonstrated that solutions of KCl, NH_4Cl, and $BaCl_2$ stimulated spawning in mantle pieces of *M. edulis*. We have also found that intact bay mussels will spawn when placed in 0.03 *M* KCl solution in sea water (unpublished observations). Sagara (1958) has shown that other bivalves can be stimulated to spawn by immersion in ammoniated sea water or by injection of NH_4OH into the gonads.

We have not been able to confirm the observations of Field (1922) and Young (1945) that rough handling, as scraping the shells, or changing the water (Just, 1939) will consistently induce spawning to any great extent. Costello *et al.* (1957) also were unable to stimulate spawning of the bay mussel by rough handling. Mild stimulation of this sort may induce spawning in a few individuals and may be the reason for the spawning in some of our control animals. It appears, however, that more severe trauma, such as partial opening of the shell, as reported here, or complete removal of the valves (Costello *et al.*, 1957), is necessary to induce spawning in a large number of animals.

Our results agree with those of Iwata (1950b) showing that electrical stimulation of intact mussels will trigger spawning; however, this stimulus is not as effective as KME.

The basis for the mechanism of spawning in mussels is not clearly understood. Iwata (1952) has shown that spawning in *M. edulis* can be induced by bathing mantle pieces in solutions of KCl, NH_4Cl, $BaCl_2$ and NH_4OH, but not in NaCl, LiCl, $SrCl_2$, $CaCl_2$ and $MgCl_2$ solutions. He explained these results on the basis of the higher mobility of the cations in the former group, and concluded that these chemical stimuli in the female do not act directly on the eggs, but rather upon the ovarian cells. The excitation of these cells then results in the maturation and subsequent release of the eggs. Lubet (1956) believes that spawning in *M. edulis* is dependent on the interaction between external and internal factors. He found that ablation of the cerebroid ganglia resulted in an accelerated spawning and concluded that the neurosecretion of these organs exerted an inhibitory action. Removal of this inhibition would then permit the animal to become receptive to the external stimuli resulting in the emission of gametes. This relationship may explain the refractoriness of certain animals subjected in our experiments, and in the experiments of others, to the external stimuli. Such stimuli then would not always act with equal efficiency on all animals.

The exact nature of the material in KME responsible for the triggering of spawning is not known. KME (combined wastes from sulfate process pulp mills) is a highly complex material and is a partial result of the alkaline digestion of wood chips. Isolation and characterization of the stimulatory material, if possible, may aid in elucidating the mechanism of spawning in mussels. Experiments bearing on this point are in progress.

It must be emphasized that stimulation of spawning in mussels by KME is a laboratory phenomenon and there is no definitive evidence that this material acts in a similar manner under natural conditions. It is unlikely that concentrations of KME in marine receiving waters would be sufficiently high to trigger spawning of mussels in their natural habitats except perhaps in the immediate vicinity of waste outfall.

Summary

1. Kraft mill effluent is a very effective material in stimulating spawning in the mussels *M. edulis* and *M. californianus*. Bay mussels obtained at all times of the year and sea mussels during January and February from the Oregon coast spawned within 24 hours after exposure to 4% KME. The gametes so obtained were viable and capable of fertilization, as shown by the artificial fertilization and development of eggs to the shell stage.

2. Electrical stimulation of intact mussels was also shown to stimulate spawning, but was not as effective as KME.

3. Mechanical stimulation, such as scraping the shells, or changing the water in which the animals were maintained, was only slightly effective in stimulating spawning. Stimulation associated with the partial opening of the valves was more effective in this regard.

4. Possible explanations for the mechanism of spawning in mussels are discussed.

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